PCT

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A01N 37/44, 33/12, A01G 7/00, C05C 11/00

A1

(11) International Publication Number: WO 96/41532

(43) International Publicati n Date: 27 December 1996 (27.12.96)

(21) International Application Number: PCT/FI95/00483

(22) International Filing Date: 7 September 1995 (07.09.95)

(30) Priority Data:
952866 9 June 1995 (09.06.95) FI

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(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, MW, SD, SZ, UG), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: IMPROVING THE YIELD OF PLANTS

(57) Abstract

The invention relates to the exogenous use of betaine to improve the yield of C-4 cereals. According to the invention, betaine is used to improve the yield especially under stress conditions. The invention also relates to C-4 cereals treated exogenously with betaine, particularly to the seeds of such plants.

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Improving the yield of plants

Technical field

The invention relates to the use of betaine to improve the yield of plants. The invention relates especially to the use of betaine to improve the yield of C-4 cereals. According to the invention, the yield can be improved both under normal and stress conditions, i.e. when the conditions are poor due to e.g. drought, high salinity, low temperatures, humidity or environmental poisons interfering with the growth. The invention also relates to C-4 cereals treated with betaine and to the parts thereof, especially seeds, and to products prepared from these.

Background

The environment and conditions of growth considerably affect the yield of plants. Optimum growth environment and conditions usually result in a yield that is large in quantity and high in quality. Under poor growth conditions both the quality and the quantity naturally deteriorate.

The physiological properties of a plant are preferably manipulated by means of breeding, both with traditional breeding methods and for example with genetic manipulation.

Several different solutions concerning cultivation technique have been developed to improve the growth conditions and yield of plants. Selecting the right plant for the right growth location is self-evident for a person skilled in the art. During the growing season plants may be protected with mechanical means by utilizing for example different gauzes or plastics or by cultivating the plants in greenhouses. Irrigation and fertilizers are generally used in order

to improve the growth. Surfactants are often used in connection with applying pesticides, protective agents and minerals. Surfactants improve the penetration of substances to plant cells, thereby enhancing and increasing the effect of the aforementioned agents and simultaneously reducing their harmful effects on the environment. However, different methods of cultivation technique are often laborious and impractical, their effect is limited (the economical size of a greenhouse, the limited protection provided by gauzes, etc.), and they are also far too expensive on a global scale. No economically acceptable chemical solutions for protecting plants from environmental stress conditions have been described so far.

Water supply is more important than any other environmental factor for the productivity of a crop, even though the sensitivity of plants to drought varies. Irrigation is usually utilized to ensure sufficient water supply. However, there are significant health and environmental problems related to irrigation, for example a sharp decrease in water resources, deterioration of water quality and deterioration of agricultural lands. It has been calculated in the field that about half of the artificially irrigated lands of the world are damaged by waterlogging and salinization. An indication of the significance and scope of the problem is that there are 255 million hectares of irrigated land in the world, and they account for 70% of the total world water consumption. In the United States alone, there are over 20 million hectares of irrigated land mainly in the area of the 18 western states and in the southeastern part of the country. They use 83% of the total water consumption for irrigation alone. It can also be noted that the use of irrigation water increases every year especially in industrial

countries. In addition to these problems, another drawback of irrigation is the high cost.

Another serious stress factor is the salinity of soil. The salinity of soil can be defined in different ways; according to the general definition, soil is saline if it contains soluble salts in an amount sufficient to interfere with the growth and yield of several cultivated plant species. The most common of the salts is sodium chloride, but other salts also occur in varying combinations depending on the origin of the saline water and on the solubility of the salts.

It is difficult for plants growing in saline soil to obtain a sufficient amount of water from the soil having a negative osmotic potential. High concentrations of sodium and chloride ions are poisonous to plants. An additional problem is the lack of minerals, which occurs when sodium ions compete with potassium ions required, however, for cell growth, osmoregulation and pH stabilization. This problem occurs especially when the calcium ion concentration is low.

The productivity of plants and their sensitivity to the salinity of soil also depend on the plant species. Halophytes require relatively high sodium chloride contents to ensure optimum growth, whereas glycophytes have low tolerance or their growth is considerably inhibited already at low salt concentrations. There are great differences even between different cultivars of a cultivated plant species. The salt tolerance of one and the same species or cultivar may also vary depending for example on the stage of growth. In the case of low or moderate salinity, the slower growth of glycophytes cannot be detected in the form of specific symptoms, such as chlorosis, but it is shown in the stunted growth of the plants and in the colour of their leaves that is darker than normal.

Moreover, the total leaf area is reduced, carbon dioxide assimilation decreases and protein synthesis is inhibited.

Plants can adapt to some extent to stress conditions. This ability varies considerably depending on the plant species. As a result of the aforementioned stress conditions, certain plants begin to produce a growth hormone called abscisic acid (ABA), which helps the plants to close their stomata, thus reducing the severity of stress. However, ABA also has harmful side effects on the productivity of plants. ABA causes for example leaf, flower and young fruit drop and inhibits the formation of new leaves, which naturally leads to reduction in yield.

Stress conditions and especially lack of water have also been found to lead to a sharp decrease in the activity of certain enzymes, such as nitrate reductase and phenylalanine ammonium lyase. On the other hand, the activity of alpha-amylase and ribonuclease increases. No chemical solutions, based on these findings, to protect plants have been described so far.

It has also been found that under stress conditions certain nitrogen compounds and amino acids, such as proline and betaine, are accumulated in the regions of growth of certain plants. The literature of the art discusses the function and meaning of these accumulated products. On the one hand it has been proposed that the products are by-products of stress and thus harmful to the cells, on the other hand it has been estimated that they may protect the cells (Wyn Jones, R.G. and Storey, R.: *The Physiology and Biochemistry of Drought Resistance in Plants*, Paleg, L.G. and Aspinall, D. (Eds.), Academic Press, Sydney, Australia, 1981).

Zhao et al. (in *J. Plant Physiol.* 140 (1992) 541 - 543) describe the effect of betaine on the cell

membranes of alfalfa. Alfalfa seedlings were sprayed with 0.2M glycinebetaine, whereafter the seedlings were uprooted from the substrate, washed free of soil and exposed to temperatures from -10°C to -2°C for one hour. The seedlings were then thawed and planted in moist sand for one week at which time regrowth was apparent on those plants that had survived. Glycinebetaine clearly improved the cold stability of alfalfa. The effect was particularly apparent at -6°C for the cold treatment. All controls held at -6°C for one hour died, whereas 67% of the seedlings treated with glycinebetaine survived.

Itai and Paleg (in *Plant Science Letters* 25 (1982) 329 - 335) describe the effect of proline and betaine on the recovery of water-stressed barley and cucumber. The plants were grown in washed sand, and polyethylene glycol (PEG, 4000 mol. wt.) was added to the nutrient solution for four days in order to produce water stress, whereafter the plants were allowed to recover for four days before harvesting. Proline and/or betaine (25 mM, pH 6.2) was sprayed on the leaves of the plant either on the first or third day of the stress or immediately before harvesting. As regards barley, it was noted that betaine supplied either before or after the stress had no effect, whereas betaine added in the end of the stress was effective. Proline had no effect. No positive effect was apparent for cucumber. On the contrary, it was found out that both betaine and proline had a negative effect.

Experiments aiming at clarifying the effects of betaine and proline on plants have thus yielded contradictory results. There are no commercial applications based on these results.

Brief description of the invention

The purpose of the present invention was to find a way to partially replace artificial irrigation

so that the amount and quality of the yield could be simultaneously ensured. Another purpose of the invention was to find a way to protect plants also under other stress conditions, such as during high salinity often connected with drought, at low temperatures, etc. Moreover, a further aim was to find a way to increase the yield under normal conditions without utilizing methods that would consume environmental resources or harm the environment.

In connection with the present invention it has now surprisingly been found that the yield of C-4 cereals can be considerably improved by means of exogenously applied betaine. Betaine has been found to be effective in improving the yield both under normal and stress conditions, and it has no such detrimental effects as the side effects of ABA. Betaine application makes it possible to considerably reduce for example the need for artificial irrigation, thus saving the environment and cutting down the costs to a great extent.

The invention thus relates to the exogenous use of betaine to improve the yield of C-4 cereals.

The invention relates especially to the use of betaine to improve the seed yield of C-4 cereals.

According to the invention, betaine is used exogenously to improve the yield of C-4 cereals both under normal and stress conditions.

The invention further relates to C-4 cereals treated exogenously with betaine and to the parts thereof, particularly ears and seeds, and to their use as such and for example in food, animal feed and forage industries.

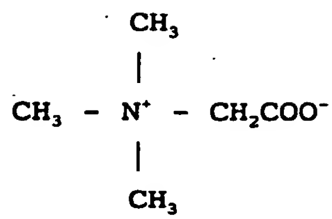
The invention also relates to a method of improving the yield of C-4 cereals, in which method betaine is exogenously applied to growing C-4 cereals.

Betaine is applied to the plant in either one or several successive treatments. The application may be performed for example by spraying together with some other spraying of for example a pesticide, if desired. Betaine used according to the invention is transported to plant cells, where it actively regulates the osmotic balance of the cells and also participates in other processes of cell metabolism. A plant cell treated with betaine is more viable even when subjected to exogenous stress factors.

The betaine treatment according to the invention is economically advantageous, and the yield increases in an amount that is economically profitable and significant. The treatment does not produce significantly more work since it may be performed together with other sprayings, and it does not require new investments in machinery, equipment or space. It should also be noted that betaine is a non-toxic natural product, which has no detrimental effects on the quality of the yield. Betaine is also a stable substance that remains in the plant cells and thereby has a long-standing effect.

Detailed description of the invention

Betaine refers to fully N-methylated amino acids. Betaines are natural products that have an important function in the metabolism of both plants and animals. One of the most common betaines is a glycine derivative wherein three methyl groups are attached to the nitrogen atom of the glycine molecule. This betaine compound is usually called betaine, glycinebetaine or trimethylglycine, and its structural formula is presented below:



Other betaines are for example alaninebetaine and prolinebetaine, which has been reported to for example prevent perosis in chicks. R.G. Wyn Jones and R. Storey describe betaines in detail in *The Physiology and Biochemistry of Drought Resistance in Plants* (Paleg, L.G. and Aspinall, D. (Eds.), Academic Press, Sydney, Australia, 1981). The publication is included herein by reference.

Betaine has a bipolar structure and it contains several chemically reactive methyl groups which it can donate in enzyme-catalyzed reactions. Most organisms can synthesize small amounts of betaine for example for the methyl function, but they cannot react to stress by substantially increasing the production and storage of betaine. Best known organisms accumulating betaine are plants belonging to the *Chenopodiaceae* family, for example sugar beet, and some microbes and marine invertebrates. The main reason for the betaine accumulation in these organisms is probably that betaine acts as an osmolyte and thus protects the cells from the effects of osmotic stress. One of the main functions of betaine in these plants and microbes is to increase the osmotic strength of the cells when the conditions require this, for example in case of high salinity or drought, thus preventing water loss. Unlike many salts, betaine is highly compatible with enzymes, and the betaine content in cells and cell organelles may therefore be high without having any detrimental effect

on the metabolism. Betaine has also been found to have a stabilizing effect on the operation of macromolecules; it improves the heat resistance and ionic tolerance of enzymes and cell membranes.

Betaine can be recovered for example from sugar beet with chromatographic methods. Betaine is commercially available from Cultor Oy, Finnsugar Bioproducts as a product that is crystalline water-free betaine. Other betaine products, such as betaine monohydrate, betaine hydrochloride and raw betaine-containing liquids, are also commercially available and they can be used for the purposes of the present invention.

According to the present invention, betaine is thus used exogenously to improve the yield of C-4 cereals, such as maize, sorghum, millet, sedge, buffalograss, crabgrass, witchgrass, etc. According to the invention, betaine is used to improve the yield of C-4 cereals both under normal and stress conditions, i.e. when the plants are subjected to periodic or continuous exogenous stress. Such exogenous stress factors include for example drought, high temperatures, high soil salinity, air pollution, such as ozone, nitric oxides, sulphur dioxide and sulphuric acid (acid rain), environmental poisons, herbicides, pesticides, etc. Treating plants subjected to stress conditions exogenously with betaine for example improves the adaptation of the plants to the conditions and maintains their growth potential longer, thereby improving the yield-producing capacity of the plants. Betaine is also a stable substance that remains in the plant cells. The positive effect of betaine is thereby long-standing and diminishes only gradually due to dilution caused by the growth.

Even though this reference and the claims use the word 'betaine', it is clear that according to the invention several different betaines can be used, if desired. It should also be noted that betaine is used here as a general term which thus covers different known betaines.

Betaine is applied to the plants in either one or several successive treatments. Application in a single dose is considered preferable. The amount used varies depending on the C-4 cereal species and cultivar, and on the stage and conditions of growth. A useful amount may be for example about 0.2 to 20 kg of betaine per hectare. A preferable amount is thus for example about 2 to 6 kg of betaine per hectare. The amounts given here are only suggestive; the scope of the present invention thus contains all amounts that work in the manner described herein.

Any method suitable for the purpose may be used for the application of betaine. Betaine can be applied separately or together with other plant protectants, pesticides or nutrients, such as fungicides and urea or micronutrients. Betaine can be applied easily for example by spraying. Foliar application of betaine and possible other agents through spraying is a preferable method which enables a more rapid response than methods involving root application. However, there may be different problems related to this method, such as low penetration concentrations in leaves with thick cuticles, run-off from hydrophobic surfaces, washing off by rain, rapid drying of the solution and leaf damage. Other methods may also be used to apply betaine, if desired.

According to the invention, an aqueous solution of betaine is preferably used.

The time of the treatment according to the invention may also vary. If betaine is applied in a single treatment, the treatment is usually performed at an early stage of growth, for example on plants of about 5 to 20 cm, or when the leaves have just come out. If betaine is applied in several successive treatments, a new spraying is performed preferably in the beginning of flowering or when stress can be forecasted on the basis of the weather.

The betaine treatment according to the invention considerably improves the yield of plants, for example the amount and quality of the yield. The treatment according to the invention can also reduce the need for artificial irrigation. The treatment according to the invention is economically advantageous and the increase in yield is economically profitable and significant. The invention has shown that for example the maize yield can be increased by over 20% with a suitable betaine dosage, for example about 6 kg/ha. It should also be noted that even though the amount of yield increases to a considerable extent, the quality does not deteriorate.

According to the invention, the yield of C-4 cereals can thus be improved both under normal and stress conditions, which in addition to drought include for example high salinity often connected with drought, high temperature, etc. Furthermore, the invention also makes it possible to grow C-4 cereals on lands that were previously considered unfit for cultivation.

The invention will be described in greater detail by means of the following examples. The examples are only provided to illustrate the invention, and they should not be considered to limit the scope of the invention in any way.

Example 1

Effect of betaine application on sorghum yield

The effect of betaine application on sorghum yield was examined at Murdoch University, Perth, Australia. The experiment was conducted under field conditions during the hot and dry summer of 1994 - 1995.

The experiment was conducted according to a split-plot design utilizing plots of 10 m^2. The plots were divided into four sub-plots that were treated with different betaine concentrations. The betaine concentrations used were 0 (control), 2 kg/ha, 4 kg/ha and 6 kg/ha. The soil was sandy (98% sand, 1% silt and 1% clay) with a low nitrogen, phosphorus and potassium content and poor water and nutrient retention properties. The amount of irrigation was normal. The cultivar was Trump. The results are shown in Table 1.

Table 1

Effect of betaine application on sorghum yield

irrigation level (%)	betaine rate (kg/ha)	sorghum seed yield (kg/ha) block I	block II	mean
100	0 (control)	2091	2201	2146
	2	2207	2302	2254
	4	2267	2354	2310
	6	2347	2435	2391

The results show that the yield increased over the control in all the experiments conducted. The best results were obtained with a betaine application rate of 4 or 6 kg/ha.

Example 2

Effect of betaine application on sorghum yield under dry conditions

The effect of betaine application on sorghum growing under water stress was examined by repeating the experiment described in Example 1, but with a 50% reduction in irrigation from the optimum amount. The results are shown in Table 2.

Table 2

Effect of betaine application on sorghum yield under water stress

irrigation level (%)	betaine rate (kg/ha)	sorghum seed yield (kg/ha)		
		block I	block II	mean
50	0 (control)	1891	1913	1902
	2	2105	2018	2061
	4	2185	2089	2137
	6	2059	2185	2122

The yield also increased clearly in this experiment compared with the control. It can also be noted that utilizing betaine according to the present invention provided similar results with a low irrigation level (50%) as with optimum irrigation. This also means that the same yield can be achieved by decreasing irrigation if a higher betaine application rate is used simultaneously.

Example 3

Effect of betaine application on maize yield

The effect of betaine application on maize yield was examined at Murdoch University, Perth, Australia. The experiment was conducted under field conditions during th hot and dry summer of 1994 - 1995.

The experiment was conducted according to a split-plot design utilizing plots of 10 m^2. The plots were divided into four sub-plots that were treated with different betaine concentrations. The betaine concentrations used were 0 (control), 2 kg/ha, 4 kg/ha and 6 kg/ha. The soil was sandy (98% sand, 1% silt and 1% clay) with a low nitrogen, phosphorus and potassium content and poor water and nutrient retention properties. The amount of irrigation was normal. The cultivar was SR-73. The results are shown in Table 3.

Table 3

Effect of betaine application on maize yield

irrigation level (%)	betaine rate (kg/ha)	maize seed yield (kg/ha) block I	block II	mean
100	0 (control)	4129	4342	4235
	2	4380	4710	4545
	4	4591	4754	4672
	6	4972	5046	5009

The results show that the yield increased over the control in all experiments. The best results were obtained with a betaine application rate of 6 kg/ha.

Example 4

Effect of betaine application on maize yield under dry conditions

The effect of betaine application on maize growing under water stress was examined by repeating the experiment described in Example 3, but with a 50% reduction in irrigation from the optimum amount. The results are shown in Table 4.

Table 4

Effect of betaine application on maize yield under water stress

irrigation level (%)	betaine rate (kg/ha)	maize seed yield (kg/ha) block I	block II	mean
50	0 (control)	3202	3204	3203
	2	3704	3882	3793
	4	3815	4484	4149
	6	4179	4394	4286

The yield also increased clearly over the control in this experiment. The best results were obtained with a betaine application rate of 4 to 6 kg/ha. It can also be noted that utilizing betaine according to the present invention produced similar results with a low irrigation level (50%) as with the optimum irrigation. This also means that the same yield can be provided by reducing irrigation if a higher betaine content is used simultaneously.

Example 5

Effect of betaine application on early development

The effect of betaine on the early development of maize was examined using water as control. The maize seeds were of the type Jubilee Hybrid Lot #1987-14, produced by Northrup King Co. Five different test solutions were prepared for the experiments as follows:

Test solution		pH
A	deionized water	7.01
B	betaine (0.02 g/l)	6.34
C	betaine (2 g/l)	6.80

The betaine was Betafin BC, Finnish Sugar Co.

Twenty maize seeds were soaked for 24 hours in 330 ml of one of the aforementioned test solutions. The seeds were then dried on stainless steel screens and sown into soil with two seeds placed in each container. The containers were then placed on a window ledge with a southern exposure to the sun, and they were watered daily with deionized water.

Ten days after the experiment began, the shoot height was measured. A second measurement was conducted 19 days after the beginning of the experiment.

The results show that betaine promoted faster germination in plants. The results are shown in Table 5.

Table 5

Effect of betaine application on the early development of maize

treatment	betaine concentration (9/1)	mean shoot height (inch)	% of the control
A control	0	2.31	100
B betaine	0.02	4.34	187
C "	2	3.16	137

Claims

1. Exogenous use of betaine to improve the yield of C-4 cereals.

2. Use according to claim 1, characterized in that betaine is used under stress conditions.

3. Use according to claim 2, characterized in that the stress conditions comprise high or low temperatures, drought or high salinity.

4. Use according to any one of claims 1 to 3, characterized in that betaine is used in an amount of about 0.2 to 20 kg/ha.

5. Use according to claim 4, characterized in that betaine is used in an amount of about 2 to 6 kg/ha.

6. A method for improving the yield of C-4 cereals, characterized in that betaine is exogenously applied to growing C-4 cereals.

7. A method according to claim 6, characterized in that betaine is applied to C-4 cereals growing under stress conditions.

8. A method according to claim 6 or 7, characterized in that the stress conditions comprise high or low temperatures, drought or high salinity.

9. A method according to any one of claims 6 to 8, characterized in that betaine is administered once or several times during the growing season.

10. A method according to any one of claims 6 to 9, characterized in that betaine is administered together with a pesticide or a surfactant.

11. A method according to any one of claims 6 to 10, c h a r a c t e r i z e d in that betaine is administered in a single treatment at an early stage of growth.

12. A method according to any one of claims 6 to 11, c h a r a c t e r i z e d in that betaine is used in an amount of about 0.2 to 20 kg/ha, preferably about 2 to 6 kg/ha.

13. A yield of C-4 cereals, especially a seed yield, obtained with the method according to any one of claims 6 to 12.

14. C-4 cereals treated exogenously with betaine, and the seeds thereof.

15. Use according to any one of claims 1 to 4, c h a r a c t e r i z e d in that the C-4 cereal is maize, sorghum or millet.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 95/00483

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A01N 37/44, A01N 33/12, A01G 7/00, C05C 11/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A01N, A01G, C05C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA, CAB ABSTRACTS, WPI, IFIPAT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 9535022 A1 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION), 28 December 1995 (28.12.95) --	1-15
X	EP 0181494 A1 (MITSUBISHI GAS CHEMICAL COMPANY, INC.), 21 May 1986 (21.05.86), page 6, compound No. 40; page 1, line 1 - line 5, page 9, line 6 - line 18; page 10, line 9 - line 14; claims 1, 5-12 --	1-15
X	STN International, File WPIDS, WPIDS accession no. 89-312201, CHIKKARIN K: "Plant supported on basal bed - is cultivated with nourishing liq. contg. betaine (s)"; JP,A,01228416, 890912 (8943 --	1-15

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
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Date f the actual completion of the internati nal search	Date of mailing of the international search rep rt
22 May 1996	24 -07- 1996
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 95/00483

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indicati n, where appropriate, f the relevant passages	Relevant to claim N .
X	JOURNAL OF EXPERIMENTAL BOTANY, Volume 38, No 188, March 1987, M. I. LONE et al, "Influence of Proline and Glycinebetaine on Salt Tolerance of Cultured Barley Embryos" page 479 - page 490 --	1-15
X	PLANT SCIENCE LETTERS, Volume 25, 1982, C. ITAI et al, "Responses of Water-Stressed Hordeum Distichum L. and Cucumis Sativus to Proline and Betaine" page 329 - page 335 --	1-15
X	J. PLANT PHYSIOL., Volume 140, 1992, Y.ZHAO et al, "Protection of Membrane Integrity in Medicago sativa L. by Glycinebetaine against the Effects of Freezing" page 541 - page 543 -- --------	1-15

INTERNATIONAL SEARCH REPORT
Information on patent family members

01/04/96

International application No.
PCT/FI 95/00483

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